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Prodrugs of 3-(3,4-dichlorobenzyloxy)-2-amino-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (MGS0039): A potent and orally active group II mGluR antagonist with antidepressant-like potential

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Abstract—3-(3,4-Dichlorobenzyloxy)-2-amino-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid **5** (MGS0039) is a highly selective and potent group II metabotropic glutamate receptor (mGluR) antagonist (antagonist activities for mGluR2; IC_{50} = 20.0 nM, mGluR3; IC_{50} = 24.0 nM) and is detected in both plasma (492 ng/mL) and brain (13.2 ng/g) at oral administration of 10 ng/mL [*J. Med. Chem.* **2004**, *47*, 4750], but the oral bioavailability of **5** was 10.9%. In order to improve the oral bioavailability of **5**, prodrugs of **5** were discovered by esterification of carboxyl group on C6-position of bicyclo[3.1.0]hexane ring. Among these compounds, 6-alkyl esters exhibited approximately 10-fold higher concentrations of **5** in the plasma and brain of rats after oral administration (e.g., ethyl ester of **5**; plasma, C_{max} = 20.7 ± 1.3 μM) compared to oral administration of **5** (plasma, C_{max} = 2.46 ± 0.62 μM). 3-(3,4-Dichlorobenzyloxy)-2-amino-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid 6-heptyl ester (**7ao**), a prodrug of MGS0039, showed antidepressant-like effects in rat forced swimming test and mouse tail suspension test following oral administration. Moreover, following oral administration of **7ao** in mice, high concentrations of MGS0039 were detected in both the brain and plasma, while **7ao** was barely detected. In this paper, we report the synthesis, in vitro metabolic stabilities, and pharmacokinetic profiles of the prodrugs of **5**, and the antidepressant-like effects of **7ao**.

1. Introduction

Glutamate is a major excitatory transmitter in the central nervous system, and glutamate receptors are classified into ionotropic and metabotropic glutamate receptors.[1,2] The metabotropic glutamate receptors (mGluRs) are coupled to G-protein. The eight mGluRs, which have been cloned and sequenced, are classified into three groups (I–III), based on their structure, intracellular signaling mechanisms, and pharmacological properties.[3–9] Group I mGluRs (mGluR1 and mGluR5) are positively coupled to phospholipase C, while group II mGluRs (mGluR2 and 3) and group III mGluRs (mGluR4, 6, 7, and 8) are negatively coupled to the activity of adenylyl cyclase.[9–11] Dysfunctional glutamatergic systems have been implicated in major psychiatric disorders such as depression, anxiety, and schizophrenia.[12] Indeed, the efficacy of group II mGluR agonists in animal models and in clinical trials suggests that the agonists may be effective in treating many diseases and conditions such as schizophrenia,[13–15] anxiety,[16–19] and panic disorder,[20] while the efficacy of antagonists for mGluRs has not been clarified in animal models or clinical trials. This might be due to the lack of potent and selective antagonists for mGluRs.

Our laboratory initiated efforts to identify potent and selective antagonists for Group II mGluRs, identifying (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyl)oxy-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid **5**[21] (MGS0039), a potent and selective antagonist for Group II mGluRs. Moreover, we found that group II mGluR

Keywords: MGS0039; Prodrug; mGluR antagonist; Metabotropic glutamate receptor; Antidepressant-like effect.
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antagonists exhibited antidepressant-like potential in experimental animal models.[22]

As shown in Chart 1, a number of antagonists of group II mGluR have been identified. Compound **1** was found to be a weak but a selective antagonist for group III mGluRs.[23] Compound **2** was a potent and relatively a selective antagonist for group II mGluRs. Of the compounds shown in Chart 1, **3**–**6** bind with very high affinity to rat brain mGluR and are potent functional antagonists for human mGluRs.[21,24–26] We recently reported on the synthesis, in vitro pharmacological profiles, and structure–activity relationships (SARs) of **5**, as well as the pharmacokinetic profiles of several analogues of **5**.[21] In particular, **5** (MGS0039) exhibited plasma and brain concentrations higher than those of (2*S*)-amino-2-((1*S*,2*S*)-2-carboxycycloprop-1-yl)-3-(9-xanthyl)propionic acid **4**[24,25] (LY341495) upon oral administration of 10 mg/kg to rats, but the oral bioavailability of **5** was merely 10.9% (**4**, <5%). The low oral bioavailability of **4** and **5** might be attributable to the low absorption from the gastrointestinal tract, possibly due to the structural feature having two carboxyl groups and an amino group. In contrast, all compounds shown in Chart 1 are modified from glutamic acid and mainly the structural feature may affect the activity as antagonists for mGluRs. In an attempt to increase the gastrointestinal absorption of **5**, prodrugs with modification to the carboxyl and amino groups in **5** were synthesized, since prodrugs had potential for good absorption and were ascribed to biologically active compound **5**.

We first designed dipeptides and esters of **5** and/or **6** and then investigated in vitro metabolic stabilities with liver S9 fraction and plasma as well as the pharmacokinetic profiles of the prodrugs. The peptidemimetic prodrugs of **5** were targeted to increase intestinal absorption via dipeptide carriers, which reports have suggested are drug delivery systems for low permeability compounds.[27] Other ester-type prodrugs more lipophilic than **5** were sought to increase absorption by passive transport.[28]

We then examined the antidepressant-like potential of **7ao**, a prodrug of MGS0039, using two behavioral despair models of depression.

In this paper, we report on synthesis, in vitro metabolic stabilities, and pharmacokinetic profiles of prodrugs of the **5** and **6**, as well as the antidepressant-like effects of **7ao**.

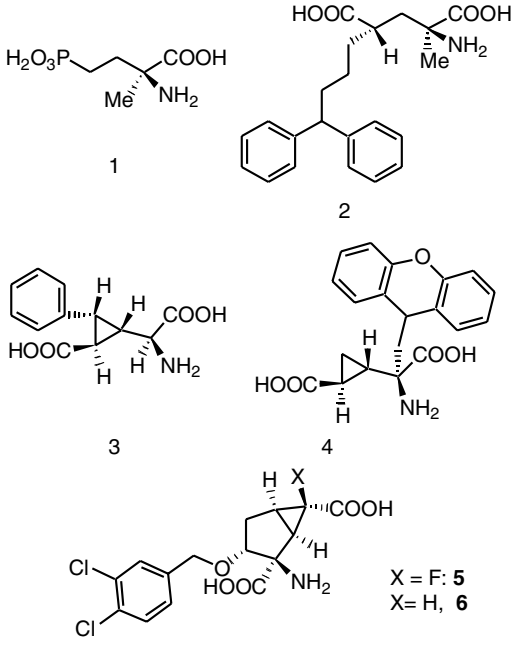


Chart 1. Group II mGluR agonist and antagonists.

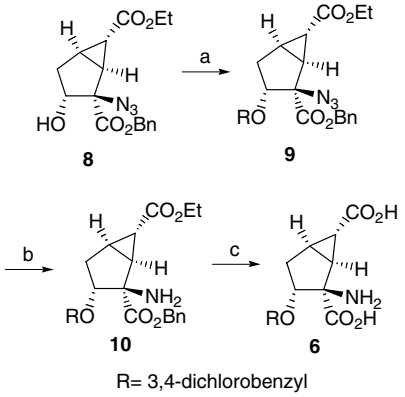
2. Chemistry

Compound **6**, the defluorine analogue of **5**, was synthesized from the intermediate **8**,[29] by the same procedure for synthesis of **5**, as shown in Scheme 1. Etherification of the 3-hydroxy group in intermediate **8** was performed using a benzyl trichloroacetimidate, which was prepared from 1,1,1-trichloroacetonitrile and 3,4-dichlorobenzyl alcohol in the presence of a catalytic amount of trifluoromethanesulfonic acid, yielding **9**.[30,31]

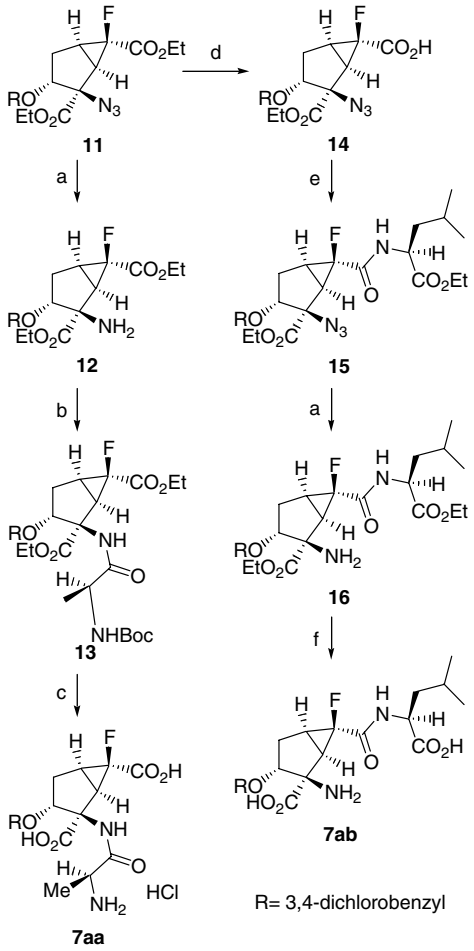
Reduction of the azide group in **9** using the Staudinger reaction[32,33] provided **10** without reductive cleavage of the benzyl group at C3-position of bicyclo[3.1.0]hexane ring. Finally, **6** was obtained by hydrolysis of two esters in **10** with LiOH.

Scheme 2 depicts the synthesis of dipeptides **7aa** and **7ab**, **5** coupled with alanine at the amino group of C2-position and with leucine at the carboxy group of C6-position in **5**.

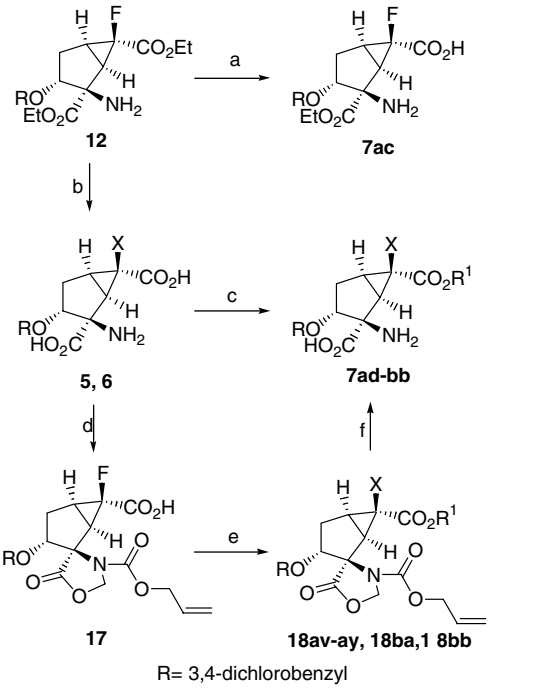
We reported that reduction of the azide group of **11**, an intermediate for synthesis of **5**, provided **12** by the Staudinger reaction.[21] The coupling reaction of **12** and a mixed anhydride of *N*-Boc-L-alanine with *i*-butyl chloroformate allowed to proceed to yield **13**. Finally, the protective groups, consisting of ethyl ester and Boc



Scheme 1. Reagents and conditions: (a) 3,4-Cl_2-$C_6H_5CH_2OC(=NH)CCl_3$, TfOH, $CHCl_3$, cyclohexane, rt (b) Me_3P, THF, H_2O, rt (c) LiOH, THF, H_2O, rt.



Scheme 2. Reagents and conditions: (a) Me_3P, THF, H_2O, rt; (b) *N*-Boc-L-alanine, *N*-methylmorphorin, *i*-Butyl chloroformate, rt; (c) (i) LiOH, THF, H_2O, rt (ii) 4N HCl, rt; (d) LiOH, THF, H_2O, 0 °C; (e) HOBT, EDC, L-leucine Et ester, *N*-methylmorphorin, 0 °C; (f) LiOH, THF, H_2O, rt.



Scheme 3. Reagents and conditions: (a) LiOH, THF, H_2O, 0 °C; (b) LiOH, THF, H_2O, rt; (c) R^1OH, $SOCl_2$. 50–70 °C (d)—(i) allyl chloroformate, dioxane, satd $NaHCO_3$, rt; (ii) paraformaldehyde, TsOH, benzene, reflux using Dean–Stark apparatus; (e) R^1Cl, NaI, K_2CO_3, DMF, 60–80 °C; (f) $Pd(PPh_3)_4$, 1,3-dimethylbarbituric acid, $CHCl_3$, 40 °C.

and ethyl ester, were sequentially removed by hydrolysis with LiOH and HCl to obtain **7aa**. Selective hydrolysis of ethyl ester at C6-position in **11** with LiOH at 0 °C provided **14** as a sole product, due to the high reactivity of ethyl ester for hydrolysis arising from the electronegativity of the fluoride atom at C6-position in **11**. The coupling reaction of **14** with L-leucine in the presence of HOBT and EDC provided **15**. According to the preparation similar to that for **7aa**, reduction of the azide group of **15** using the Staudinger reaction and hydrolysis of ethyl ester **16** with basic conditions provided dipeptide **7ab**.

The synthesis of esters **7ac**–**bb**, monoesters at C2- and C6-position, from **5** or **6** is shown in Scheme 3.

Compound **7ac**, a monoester at C2-position in **5**, was synthesized from **12**[21] by selective hydrolysis of ethyl ester, according to the preparation of monoester **14**, the first step of synthesis of **7ab**. Selective esterification of **5** with alkyl alcohol and benzyl alcohol as a solvent was performed in the presence of $SOCl_2$ at 50–80 °C to obtain the corresponding monoesters **7ad**–**au** or **7az** at C6-position in **5** or **6** as a single product. Compounds **7av**–**ay**, **7ba**, and **7bb** required protection of α-amino acid moiety at C2-position in **5** or **6**, since directed esterification of **5** with alkoxycarbonyloxyalkyl, butyroyloxymethyl, and 2-morphorin-4-ylethyl halide under basic conditions resulted in diesters. Selective α-carbonyl-protecting methods with 5-oxazolidinone derivative[34–36] were adopted for syntheses of compounds **7av**–**ay**, **7ba**, and **7bb**.[34–36] Compound **5** was allowed to react with allyl chloroformate, an amine-protective agent, in the presence of aqueous sodium bicarbonate in dioxane to produce the corresponding allyl carbamate. Then the reaction of the allyl carbamate with paraformaldehyde in the presence of *p*-toluenesulfonic acid as a catalyst was carried out using the Dean–Stark apparatus to obtain **17**. An esterification of **17**, having the protecting group on α-amino acid moiety, with alkoxycarbonyloxyalkyl, butyroyloxymethyl, and 2-morphorin-4-ylethyl halide, and excess amount of K_2CO_3 provided **18av**–**ay**, **18ba**, and **18bb**. Cleavage of the protective groups of **18av-ay**, **18ba**, and **18bb** with $Pd(PPh_3)_4$ and barbituric acid[37] yielded **7av**–**ay**, **7ba**, and **7bb**.

3. Results and discussion

3.1. Metabolic stability study

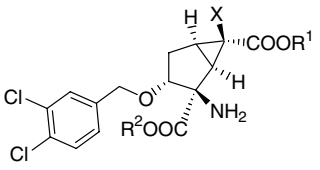
We first investigated the in vitro metabolism of the prodrugs of **5** or **6** using plasma and liver S9 fraction of rat.

Table 1 shows the transformed ratio from prodrugs to **5** or **6**. Following incubation of dipeptides **7aa** and **7ab**, compounds with an alanine and a leucine incorporated at the C2-position and C6-position of bicyclo[3.1.0]hexane ring, no transformation to **5** was observed. The incubation of **7ac**, ethyl ester at the 2-position, with plasma and S9 fraction of rat also failed to yield **5**.

On the other hand, all compounds of alkyl and benzyl esters (**7ad-as** and **7au**) at the C6-position of bicyclo[3.1.0]hexane ring yielded **5** when incubated with rat plasma and/or liver S9.

The ratio of hydrolysis of **7az** (plasma; 17.7%, S9; 17.8%), an ethyl ester of **6** (defluorine analogue of **5**), was lower than that of **7ae** (plasma; 88.7%, S9; 95.9%) under the same incubation conditions. All of **7av**, **7aw**, **7ba**, and **7bb**, of which esters were activated for enzymatic hydrolysis and the commonly employed in prodrug moieties for acid-derived drugs,[38–40] transformed into biologically active substances **5** in high ratio (>95%). These findings suggest that the fluorine atom at the C6-position of bicyclo[3.1.0]hexane ring plays an important role in the hydrolysis of alkyl esters. We infer that the high electron-negativity of fluorine atom results in the high acidity of carboxylic acid at the C6-position of **5**, resulting in ready hydrolysis of the esters using plasma and liver S9 fractions.

Table 1. The ratios of hydrolysis of **7aa**–**bb** to **5** or **6**



7	R^1	R^2	X	Plasma	Liver S9
aa	H	Ala	F	1.2	<1.0
ab	Leu	H	F	0.2	<1.0
ac	H	Et	F	0.2	<1.0
ad	Me	H	F	87.8	82.6
ae	Et	H	F	88.7	95.9
af	*n*-Propyl	H	F	91.4	100
ag	*i*-Propyl	H	F	88.1	100
ah	*n*-Butyl	H	F	91.2	100
ai	*i*-Butyl	H	F	89.7	100
aj	*n*-Pentyl	H	F	62.9	100
ak	3-Methylbutyl	H	F	100.0	93.1
al	*n*-Hexyl	H	F	100.0	99
am	4-Methylpentyl	H	F	NT	99.1
an	Cyclohexyl	H	F	95.6	100
ao	*n*-Heptyl	H	F	NT	98
ap	5-Methylhexyl	H	F	NT	94.6
aq	Cyclohexylmethyl	H	F	33.7	99.1
ar	*n*-Octyl	H	F	NT	94.9
as	6-Methylheptyl	H	F	NT	90.6
at	*n*-Decyl	H	F	NT	NT
au	Benzyl	H	F	95.6	100
av	CH(Me)OCOOEt	H	F	98.2	100
aw	CH(Me)OCOO-cyclohexyl	H	F	96.7	100
ax	$CH_2OCO(CH_2)_2Me$	H	F	NT	NT
ay	2-Morphorin-4-ylethyl	H	F	54.9	95.4
az	Et	H	H	17.7	17.8
ba	CH(Me)OCOOEt	H	H	100.0	97.7
bb	CH(Me)OCOO-cyclohexyl	H	H	100.0	100

NT, not tested.

3.2. Plasma concentration profiles and brain penetration in rats

We investigated the bioavailability of **7aa**–**ac** and **7ae** to determine the suitable chemical modifications (positions and substitutions) for prodrugs in in vivo studies. Bioavailability studies of **7aa**–**ac** and **7ae** were performed in fasting Wistar rats, and the pharmacokinetic parameters are shown in Table 2.

Following intravenous administration of **5** at 1 mg/kg, the plasma clearance was 176.3 mL/h/kg and the volume of distribution was 302.8 mL/kg. The values for half-life ($t_{1/2}$) and AUC_{inf} were 2.9 h and 15.2 μM h, respectively. Oral bioavailability studies in fasting rats were performed at 10 mg/kg. Plasma concentration reached maximum levels (C_{max}) of 2.5 μM at 2.7 h, declining thereafter with a $t_{1/2}$ of 2.8 h. The value of AUC_{inf} was 16.6 μM h, and absolute bioavailability was 10.9%. This low bioavailability is believed to be attributable to low absorption rather than the first pass effect, since **5** is stable in rat liver S9 fractions.

After oral administration of **7aa**–**ac** and **7ae** at a dose of 10 mg/kg, plasma concentrations of **5** reached C_{max} of 0.01, 0.3, 1.2, and 20.7 μM at 4.0, 3.3, 4.0, and 1.0 h, respectively. The values of AUC_{inf} were 0.1, 2.0, 23.8, and 86.2 μM h, and figures for oral bioavailability determined by the AUC_{inf} values following intravenous dosing of **5** were 0.1%, 1.7%, 16.8%, and 66.6%, respectively.

Compared to the three other prodrugs, compound **7ae** exhibited the highest plasma concentration of **5** at the early sampling time points for both intravenous and oral administration, with oral bioavailability of biologically active substance **5** improving from 10.9% to 66.6%. These findings suggest that **7ae** was absorbed rapidly and hydrolyzed effectively as indicated in in vitro experiments with rat plasma and liver S9 fractions.

The penetration of **5** into the brain relative to that in plasma was examined in fasting Wistar rats following oral doses of 10 mg/kg of **5** and **7aa**–**ac** and **7ae**. As shown in Table 3, after oral dosing of **5**, the brain levels were 0.02 and 0.03 μM at 3 and 6 h post-dose, respectively. The corresponding plasma levels were 1.13 and 1.30 μM, and brain to plasma concentration ratios were 0.03 in both time points. Following oral dosing of **7ae**, the increasing of brain levels of **5** was observed. These levels were 0.30 and 0.25 μM at 3 and 6 h post-dose, respectively. The corresponding plasma levels were 16.28 and 4.75 μM, and brain/plasma concentration ratios were 0.02 and 0.05, indicating that elevated levels in the brain were due to higher plasma levels of **5**, since brain/plasma concentration ratios were virtually the same as dosing of **5**. On the other hand, following oral dose of **7aa**–**ac**, increasing brain levels were not observed.

Table 2. Pharmacokinetic parameters of **5** after intravenous (1 mg/kg) and oral dosing (10 mg/kg) of prodrugs (**7aa**–**ae**) to Wistar rats

Dosed compound	Route	Dose (μmol/kg)	T_{max} (h)	C_{max} (μM)	$t_{1/2}$ (h)	AUC_{inf} (μM h)	F (%)
7aa	iv	2.1	1.2 ± 0.8	0.03 ± 0.02	11.5 ± 12.9	0.3 ± 0.2	
7ab	iv	2.0	1.7 ± 2.1	0.04 ± 0.01	8.7 ± 4.6	0.1 ± 0.0	
7ac	iv	2.5	2.0 ± 0.0	0.4 ± 0.10	4.0 ± 0.8	3.5 ± 0.7	
7ae	iv	2.3	0.03 ± 0.0	40.2 ± 6.96	1.6 ± 0.2	19.5 ± 1.5	
7aa	po	20.6	4.0 ± 0.0	0.01 ± 0.00	5.4 ± 1.2	0.1 ± 0.1	0.1
7ab	po	20.4	3.3 ± 1.2	0.3 ± 0.2	3.5 ± 1.3	2.0 ± 0.8	1.7
7ac	po	24.6	4.0 ± 0.0	1.2 ± 0.6	12.4 ± 4.0	23.8 ± 10.9	16.8
7ae	po	22.6	1.0 ± 0.0	20.7 ± 1.3	2.0 ± 0.4	86.2 ± 6.9	66.6

Results are expressed as the mean ± SD of three animals.

Table 3. Brain penetration of **5** after oral dosing of **5** and its prodrug (10 mg/kg) to fasting Wistar rats

Time (h)	Dosed compound	Dose (μmol/kg)	Plasma (μM)	Brain (μM)	Brain/plasma
3	**5**	26.4	1.13 ± 0.08	0.02 ± 0.01	0.03 ± 0.01
	7aa	20.6	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	7ab	20.4	0.41 ± 0.30	0.00 ± 0.00	0.00 ± 0.00
	7ac	24.6	1.62 ± 0.42	0.02 ± 0.00	0.01 ± 0.00
	7ae	22.6	16.28 ± 0.95	0.30 ± 0.03	0.02 ± 0.00
6	**5**	26.4	1.30 ± 0.91	0.03 ± 0.02	0.03 ± 0.01
	7aa	20.6	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	7ab	20.4	0.23 ± 0.11	0.00 ± 0.01	0.01 ± 0.03
	7ac	24.6	1.17 ± 0.53	0.03 ± 0.01	0.03 ± 0.00
	7ae	22.6	4.75 ± 0.99	0.25 ± 0.01	0.05 ± 0.01

Results are expressed as means ± SD of three animals.

In the studies above, **7ae** showed the highest improvement in oral bioavailability, and this result well corresponded with the fact of efficient enzymatic hydrolysis in the in vitro study. Moreover, an approximately 10-fold improvement was obtained in brain levels of **5**. In contrast, no improvement in oral bioavailability or brain levels was observed following oral dosing of **7aa**–**ac**. These results are consistent with the resistance to enzymatic hydrolysis observed in the in vitro study. Based on these pharmacokinetic profiles of four prodrugs and in vitro experiments, it may be difficult to improve the oral bioavailability of **5** via esters at the C2-position and derivatives of **5** modified with amino acids. Thus, we focused our examination on esters on the C6-position of bicyclo[3.1.0]hexane ring.

Oral bioavailability studies of alkyl esters **7ad**–**bb** were performed in rats at doses of 10 mg/kg. The pharmacokinetic parameters are shown in Table 4.

Compounds **7ad**–**at**, alkyl esters, were administered at a dose of 10 mg/kg to fasting rats. Plasma concentrations of **5** reached peak levels within 0.5–1.5 h. Bioavailabilities based on plasma concentration of 5, for **7ad**, **7ag**, and **7ai**, were >70% with C_{max} values of 22.9–28.9 μM. The next highest values for bioavailability (50.7–67.4%) were obtained in **7af**, **7ah**, **7aj**, **7am**, **7an**, and **7aq** with C_{max} values of 15.5–24.8 μM. Brain levels were 0.19–0.24 μM at 6 h. All alkyl esters were completely hydrolyzed by liver S9 fractions as well as plasma in rats with improved bioavailability and increase in brain levels.

In the case of **7au**, a benzyl ester, plasma concentrations of **5** reached the maximum levels at 1.3 h with a C_{max} value of 13.2 μM and bioavailability was 41.5%.

In cases of **7av** and **7aw**, bioavailabilities based on plasma concentration of **5** were 12.2% and 12.7%, respectively. Compound **7ay**, a substituted alkyl ester, was administered at a dose of 10 mg/kg to fasting rats. Plasma concentrations of **5** reached peak levels at 2.3 h with the C_{max} value of 0.5 μM. Bioavailability was a mere 2.0%. Compounds **7au**–**aw** and **7ax** completely transformed into **5** in in vitro experiments, and large non-enzymatic degradations were observed. These products, which are alkoxycarbonylethyl esters, did not show improved bioavailability for **5**, in spite of ensuring stability in dosing solution. These findings suggest that resistance to chemical degradation as well as high hydrolysis rate in liver S9 and plasma are required to improve bioavailability of **5**.

We next investigated **7az**–**bb**, prodrugs of **6** (defluorine analogue of **5**).

We examined the pharmacokinetic profiles of **6**, which exhibited high affinity for mGluR2 (K_i = 2.54 nM) and potent antagonist activity for mGluR2 (IC_{50} = 34.2 nM) as well as **5**.[41] Following intravenous administration of 1 mg/kg **6** to non-fasting rats, plasma levels appeared to decline biphasically with $t_{1/2}$ of 4.3 h and total plasma clearance of 258.4 mL/h/kg. The volume of distribution was estimated at 446.4 mL/kg, and the AUC value was 10.8 μM h. Following oral administration of 10 mg/kg **6** to fasting rats, plasma concentrations reached a C_{max} of 0.4 μM at 4.7 h, declining thereafter with a $t_{1/2}$ of 3.8 h. The AUC_{inf} value was 3.8 μM h, and absolute bioavailability was 3.6%. We also investigated the prodrugs of **6** to improve the oral bioavailability of **6** based on the successful results with prodrugs for **5**.

Table 4. Pharmacokinetic parameters and brain levels of **5** and **6** after oral dosing (10 mg/kg) of **7ad**–**bb** to male Sprague–Dawley or Wistar rats

Dosed compound	Dose (μmol/kg)	T_{max} (h)	C_{max} (μM)	$t_{1/2}$ (h)	AUC_{inf} (μM h)	F[a] (%)	Brain at 6 h (μM)
7ad	23.3	1.0 ± 0.0	28.9 ± 7.2	1.4 ± 0.2	90.0 ± 18.1	70.6	0.20 ± 0.01
7af	21.9	1.0 ± 0.0	24.8 ± 6.2	1.6 ± 0.1	80.6 ± 12.8	67.4	0.23 ± 0.03
7ag	21.9	1.0 ± 0.0	22.9 ± 2.1	2.0 ± 0.8	88.3 ± 6.4	73.9	0.25 ± 0.03
7ah	21.2	1.3 ± 0.6	17.0 ± 0.5	1.8 ± 0.1	63.7 ± 1.8	54.9	0.24 ± 0.03
7ai	21.2	0.8 ± 0.3	24.1 ± 6.6	1.5 ± 0.3	86.0 ± 13.6	74.1	0.26 ± 0.08
7aj	22.3	1.3 ± 0.6	23.9 ± 4.0	1.1 ± 0.1	72.0 ± 9.7	59.1	0.23 ± 0.05
7ak	22.3	1.3 ± 0.6	17.8 ± 5.4	1.4 ± 0.4	54.4 ± 18.8	44.7	0.20 ± 0.05
7al	21.6	1.0 ± 0.0	20.8 ± 5.2	1.2 ± 0.3	54.9 ± 4.6	46.5	0.21 ± 0.01
7am	21.6	0.8 ± 0.3	18.3 ± 5.0	2.0 ± 0.9	59.9 ± 10.2	50.7	0.19 ± 0.01
7an	20.1	1.0 ± 0.0	16.6 ± 3.1	1.7 ± 0.3	59.9 ± 15.6	54.5	0.22 ± 0.05
7ao	21.0	1.0 ± 0.0	14.9 ± 3.7	1.2 ± 0.2	45.4 ± 14.9	39.6	0.19 ± 0.01
(**7ao**	21.0	1.3 ± 0.6	12.9 ± 0.9	2.0 ± 0.1	105.4 ± 27.4	73.0[b]	
7ap	21.0	0.5 ± 0.0	17.3 ± 1.3	1.6 ± 0.5	45.1 ± 6.0	39.4	0.15 ± 0.04
7aq	19.6	1.3 ± 0.6	15.5 ± 3.2	1.5 ± 0.6	61.5 ± 20.3	57.5	0.19 ± 0.01
7ar	20.4	1.0 ± 0.0	17.7 ± 4.2	1.2 ± 0.2	48.6 ± 8.3	43.6	0.19 ± 0.01
7as	21.6	0.7 ± 0.3	15.0 ± 1.2	1.6 ± 0.4	42.9 ± 7.3	36.3	0.18 ± 0.04
7at	19.3	0.8 ± 0.3	14.5 ± 0.9	1.1 ± 0.1	43.2 ± 3.8	41.0	0.18 ± 0.21
7au	21.4	1.3 ± 0.6	13.2 ± 2.4	1.4 ± 0.1	48.3 ± 11.2	41.5	0.19 ± 0.03
7av	20.2	1.0 ± 0.0	5.3 ± 3.1	1.2 ± 0.1	13.5 ± 7.4	12.2	0.07 ± 0.03
7aw	18.2	1.0 ± 0.0	4.2 ± 0.7	1.0 ± 0.1	12.6 ± 1.3	12.7	0.06 ± 0.00
7ax				NT			
7ay	17.7	2.3 ± 1.5	0.5 ± 0.1	1.1 ± 0.5	1.9 ± 0.4	2.0	0.00 ± 0.00
7az	23.5	2.0 ± 0.0	3.7 ± 0.2	1.9 ± 0.6	18.5 ± 1.9	20.2	0.09 ± 0.02
7ba	21.0	1.7 ± 0.6	2.8 ± 0.9	1.2 ± 0.3	9.8 ± 2.2	11.9	0.07 ± 0.01
7bb	18.9	1.3 ± 0.6	1.9 ± 1.4	1.8 ± 1.1	6.8 ± 4.3	9.2	0.05 ± 0.04
5	26.4	2.7 ± 1.2	2.5 ± 0.6	2.8 ± 0.1	16.6 ± 6.1	10.9	0.03 ± 0.02
6	27.8	4.7 ± 3.1	0.4 ± 0.1	4.0 ± 0.5	3.8 ± 0.2	3.6	0.01 ± 0.00

Results are expressed as means ± SD of three animals. **7ad**–**7bb**: Sprague–Dawley rats; **5** and **6**: Wistar rats; NT, not tested.

[a] $F = (AUC_{inf}/dose)/(AUC_{inf}, iv/dose, iv) \times 100$.

[b] The oral bioavailability was evaluated by adding the sampling points up to 24 h.

After oral administration of 10 mg/kg **7az**–**bb** to fasting rats, the plasma concentration of **6** for each prodrugs reached the maximum levels of 3.7, 2.8, and 1.9 μM at 2.0, 1.7, and 1.3 h, respectively. The corresponding AUC_{inf} values were 18.5, 9.8, and 6.8 μM h, and bioavailabilities of the active form were 20.2%, 11.9%, and 9.2%, respectively. Plasma concentrations of **6** reached peak levels of 4.8, 0.0, and 0.0 μM at 0.5, 0.8, and 0.8 h, respectively.

Compound **7ae**, an ethyl ester of **5**, was almost completely hydrolyzed by rat liver S9 fractions and plasma, but **7az**, an ethyl ester of **6**, was stable in liver S9 fractions and plasma. Moreover, **7ae** exhibited increased the bioavailability of **5** to 66.6% in rats, while **7az** increased the bioavailability only to 20%. Based on the improved bioavailability in ester-type prodrugs related to the hydrolysis rate of prodrugs in liver S9 fractions and plasma, the fluoride atom in **7ad**–**ay** appears to play an important role in hydrolysis. Ester-type prodrugs improved the bioavailability of **5** having fluoride atom at the C6-position of the bicyclo[3.1.0]hexane ring.

In the case of **7av** and **7aw**, which are alkoxycarbonyloxyethyl esters of **5**, we observed non-enzymatic degradation with 40–80%, although they were completely transformed to **5** in liver S9 fractions. We had assumed that **7av** and **7aw** might have been transformed into **5** in the gastrointestinal tract due to low stabilities for chemical degradation and the hydrolysis has given the low bioavailability of **5** comparing with the alkyl ester-type prodrug **7ad**–**at** of **5**. In contrast, **7ba** and **7bb** were stable against chemical degradation and were completely hydrolyzed by liver S9 fractions. However, the stability did not improve bioavailability. These findings suggest the need for further investigation of the pH range for chemical stability, stability in the absorption phase (in intestinal microsomes and/or S9 fractions), penetration into absorption sites, and other related issues.

Finally, we confirmed that **7ao** was not a ligand for mGluR2. The affinity of **7ao** was evaluated by [^{3}H]-(1*S*,2*S*,3*S*,5*R*,6*S*)-2-amino-3-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid binding using CHO cells stably expressing mGluR2.[13] Compound **7ao** did not exhibit significant affinity for mGluR2 (**7ao**: K_i = >1000 nM, **5**: K_i = 2.38 nM[21]). These findings suggest that **7ao** is not a ligand for mGluR2 and acts as a prodrug of **5**.

3.3. Antidepressant-like effects of the prodrug (7ao) of 5

We examined the antidepressant potential of **7ao**, a prodrug of **5**, in two despair behavioral models of depression.

3.4. Forced swimming test in rats

Oral administration of **7ao** dose-dependently and significantly reduced immobility time with the lowest active dose of 3 mg/kg (Fig. 1a). According to the behavioral scoring method reported by Detke et al.,[42] **7ao** significantly increased swimming behavior without changing

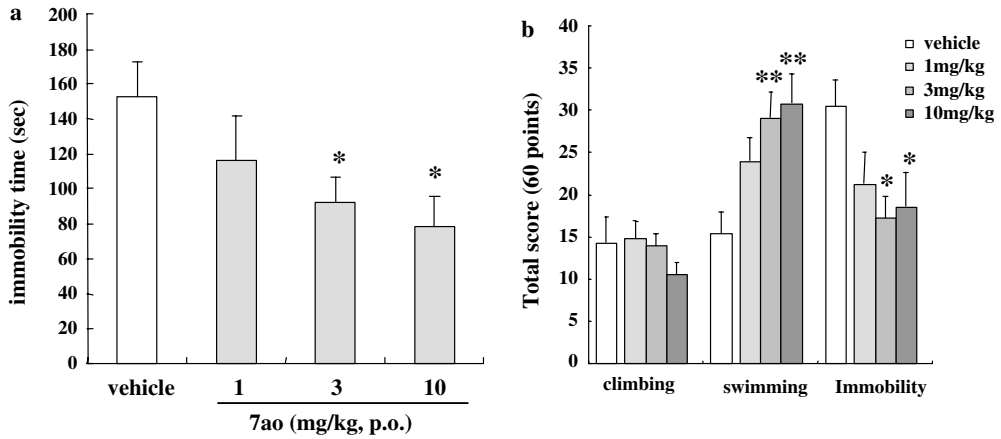


Figure 1. Effect of **7ao** in forced swimming test in rats. The effect of **7ao** was evaluated by both the method (a) duration of immobility and by the time-sampling technique (b) described by Detke et al. (1995). Data represent means ± SEM ($n = 8$). *$P < 0.05$, **$P < 0.01$ versus vehicle-treated group (Dunnett's test).

climbing behavior (Fig. 1b), similar to the findings for **5** and fluvoxamine.[21]

3.5. Tail suspension test in mice

Compound **7ao** showed dose-dependent and significant antidepressant-like effects on the mouse tail suspension test following oral administration (Fig. 2).

After 10 min test session, mice were sacrificed, and concentrations of **5** and **7ao** in the brain and plasma were measured. High concentrations of **5** were detected in both the brain and plasma, while **7ao** was barely detected (Table 5).

3.6. Effect on spontaneous locomotor activity

Compound **7ao** did not affect spontaneous locomotor activity in rats at 3 and 10 mg/kg, po, while **7ao** significantly increased locomotor activity at 30 mg/kg, po (Fig. 3). In contrast, oral administration of **7ao** did not affect spontaneous locomotor activity up to 30 mg/kg in mice (Fig. 3).

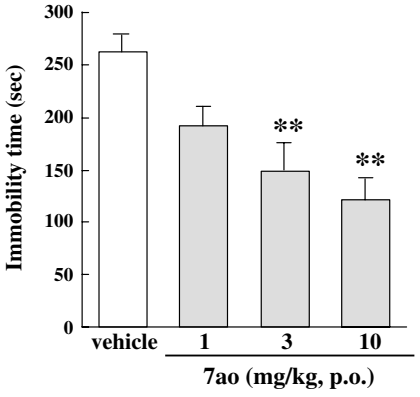


Figure 2. Effect of **7ao** in tail suspension test in mice. Compound **7ao** was administered po 2 h prior to the test. Data represent means ± SEM ($n = 15$). **$P < 0.01$ versus vehicle-treated group (Dunnett's test).

Table 5. Mean plasma and brain levels (μM) of **5** and **7ao** after oral dosing of **7ao** to male ICR mice at a dose of 1, 3, and 10 mg/kg

Dose (mg/kg)	5		7ao	
	Plasma	Brain	Plasma	Brain
1	0.52 ± 0.35	0.04 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
3	1.03 ± 0.31	0.10 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
10	5.51 ± 2.79	0.36 ± 0.13	0.01 ± 0.01	0.00 ± 0.00

Results are expressed as means ± SD of 15 animals.

Oral administration of **7ao** exhibited significant antidepressant-like effects in two behavior despair models, the forced swimming test and the tail suspension test, both of which are indicative of antidepressant-like potential in humans. Compound **7ao** showed antidepressant-like effects at doses, which did not affect locomotor activity, indicating that antidepressant-like effects are not ascribed to altered locomotion.

Moreover, following oral administration of **7ao** in mice, a large amount of **5** was detected in both brain and plasma (at rats more than 10-fold that of the IC_{50} value), while **7ao** was barely detected.

These results indicate that **7ao**, a prodrug of **5**, shows antidepressant-like effects in rodent models of depression following oral administration, and that these effects are attributable to **5**.

4. Conclusion

This paper presented in vitro metabolic stabilities and pharmacokinetic profiles for prodrugs of 3-(3,4-dichlorobenzyloxy)-2-amino-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid **5** (MGS0039) and 3-(3,4-dichlorobenzyloxy)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid **6** and antidepressant-like effects of **7ao**. Of the prodrugs presented, the alkyl esters at the C6-position of the bicyclo[3.1.0]hexane ring exhibited approximately 10-fold higher concentrations of MGS0039 in plasma and brain, while most alkyl esters were not

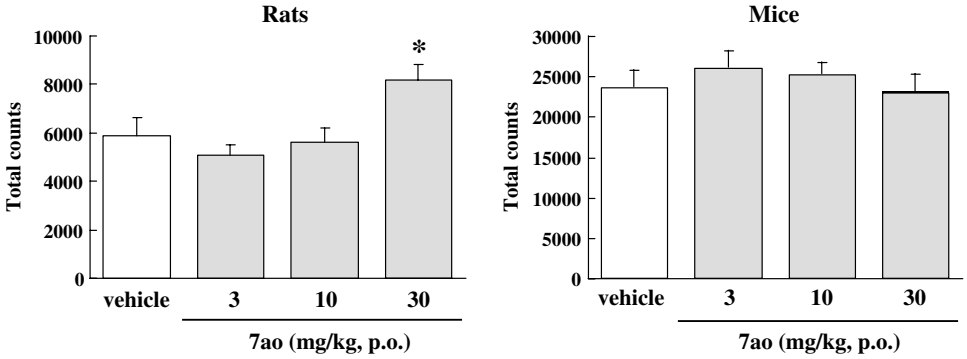


Figure 3. Effect of **7ao** on spontaneous locomotor activity in rats and mice. Compound **7ao** was administered po 2 h prior to the test. Data represent means ± SEM (n = 8 for rats, 10 for mice). *P < 0.05 versus vehicle-treated group (Dunnett's test).

detected. In contrast, the prodrugs of **6** exhibited poor pharmacokinetic profiles. These findings indicate that the fluorine atom at the C6-position of the bicyclo[3.1.0]hexane ring plays an important role in improving pharmacokinetic profiles, especially for hydrolysis of alkyl ester-type prodrugs.

Compound **7ao**, a prodrug of MGS0039, showed antidepressant-like effects in rat forced swimming test and mouse tail suspension test following oral administration, effects attributable to MGS0039.

5. Experimental

5.1. Chemistry

Melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained using a Varian Gemini 2000 (200 MHz), or Varian Unity Inova 300 (300 MHz), JEOL Alpha500 or JEOL Lambda500. Chemical shifts are reported in parts per million relative to tetramethylsilane (TMS) or sodium 3-trimethylsilylpropionate-2,2,3,3-D4 (TMSP) as an internal standard. Mass spectra (MS) were obtained on a JEOL JMS-SX102 (FAB) or Micromass Platform LC (Ion Spray). Optical rotations were determined with a JASCO DIP-360 polarimeter and are reported at the sodium D-line (589 nm). Elemental analyses were performed using a Perkin-Elmer 2400. Silica gel (C-200, 100–200 mesh (Wako Pure Chemical)) was used for column chromatography, using the solvent systems (volume ratios) indicated below.

5.1.1. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Azido-3-(3,4-dichlorobenzyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl 6-ethyl ester (9). A THF (153 mL) solution of 3,4-dichlorobenzyl alcohol (1.04 g, 9.62 mmol) was added to a Et_2O (2 mL) suspension of NaH (60%, 38 mg, 0.962 mmol) and stirred for 0.5 h at room temperature. Trichloroacetonitrile (0.96 mL, 9.62 mmol) was added to the mixture with ice-cooling and stirred for 1.5 h at room temperature. Pentane (1 mL) and methanol (37 μL) were added to the reaction mixture. After stirring for 30 min at room temperature, the formed precipitate was filtered off. The filtrate was concentrated under reduced pressure to yield crude 3,4-dichlorobenzyl-2,2,2-trichloroacetoimidate (2.38 g) as a brown viscous liquid.

Trifluoromethane sulfonic acid (360 μL) was added to the mixture of 3,4-dichlorobenzyl-2,2,2-trichloroacetoimidate (6.77 g, 21.1 mmol), **8** (5.10 g, 14.0 mol), CH_2Cl_2 (18 mL), and cyclohexane (36 mL) under a nitrogen atmosphere. The mixture was then stirred for 1 h at room temperature. Trifluoromethane sulfonic acid (360 μL) was added to the mixture, which was stirred for 2 h, after which the precipitate was filtered off. Saturated $NaHCO_3$ was added to the filtrate with ice-cooling. The mixture was extracted with $CHCl_3$, and the $CHCl_3$ layer was washed with saturated brine, dried with Na_2SO_4, concentrated under reduced pressure, and chromatographed (hexane/AcOEt 15:1) to yield **9** (2.36 g, 32%) as a colorless oil. ^{1}H NMR (200 M Hz, $CDCl_3$, TMS) δ 1.26 (3H, t, J = 7.0 Hz), 1.75 (1H, t, J = 3.1 Hz), 2.02–2.36 (4H, m), 3.55 (1H, dd, J = 8.8, 7.5 Hz), 4.13 (2H, q, J = 7.0 Hz), 4.34 (1H, d, J = 12.3 Hz), 4.51 (1H, d, J = 12.3 Hz), 5.22 (1H, d, J = 11.9 Hz), 5.34 (1H, d, J = 11.9 Hz), 7.01 (1H, dd, J = 8.1, 2.0 Hz), 7.22–7.47 (7H, m); MS (ion spray) (Positive) m/z; 526 $(M+Na)^+$; $[\alpha]_D^{26}$ +7.83 (c 3.5, $CHCl_3$).

5.1.2. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-(3,4-dichlorobenzyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl 6-ethyl ester (10). A mixture of **9** (2.31 g, 4.42 mmol), Me_3P (1 M THF solution, 4.86 mL), THF (64 mL), and H_2O (6.4 mL) was stirred for 12 h at room temperature. Ether was added to the reaction mixture, and the organic layer of the mixture was separated. The organic layer was washed with saturated $NaHCO_3$ and saturated brine, dried with Na_2SO_4, evaporated, and chromatographed (hexane/AcOEt 4:1–1:1) to yield **10** (1.51 g, 69%) as a pale yellow oil. ^{1}H NMR (200 MHz, TMS, $CDCl_3$) δ 1.24 (3H, t, J = 7.3 Hz), 1.73 (1H, t, J = 3.1 Hz,), 1.95–2.33 (4H, m), 3.47 (1H, dd, J = 8.8, 7.0 Hz), 4.10 (4H, q, J = 7.3 Hz), 4.37 (1H, d, J = 12.3 Hz), 4.46 (1H, d, J = 12.3 Hz), 5.18 (1H, d, J = 12.3 Hz), 5.30 (1H, d, J = 12.3 Hz), 7.01 (1H, dd, J = 8.4, 2.2 Hz), 7.29–7.38 (7H, m); MS (ion spray) (Positive) m/z 501 (M^++Na); $[\alpha]_D^{27}$ +15.9 (c 5.7, $CHCl_3$).

5.1.3. (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-(3,4-dichlorobenzyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (6). A mixture of **10** (1.48 g, 2.98 mmol), THF (27 mL), H_2O (13.5 mL), and $LiOH \cdot H_2O$ (345 mg, 8.2 mmol) was stirred for 4 days at room temperature. The reaction mixture was acidified with 1 N HCl and stirred for 1 h at room temperature. The solution was chromatographed on AG50W-X8 cation-exchange resin (Bio-Rad) (H_2O–50% aqueous THF–10% aqueous pyridine) to yield **6** (980 mg, 88%) as a white powder. Mp > 250 °C (decomp.); ^{1}H NMR (300 MHz, D_2O, TMSP) δ 1.61 (1H, t, J = 3.0 Hz), 1.97–2.41 (4H, m), 3.78 (1H, dd, J = 8.5, 7.9 Hz), 4.50 (1H, d, J = 12.1 Hz), 4.55 (1H, d, J = 12.1 Hz), 7.27–7.31 (1H, m, J = 12.1 Hz), 7.53–7.58 (2H, m); MS (ion spray) (Negative) m/z 358 (M^--1); $[\alpha]_D^{28}$ +5.1 (c 2.0, 1 N NaOH).

5.1.4. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-((2S)-2-*tert*-Butoxycarbonylaminopropionylamino)-3-(3,4-dichlorobenzyloxy)-6- fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid diethylester (13). *N*-Methylmorpholin (184 mL, 1.67 mmol) and *i*-butyl chloroformate (218 μL, 1.67 mmol) were added to a CH_2Cl_2 solution of *N*-Boc-L-alanine (316 mg, 1.67 mmol) at −14 °C under a nitrogen atmosphere. After dropwise addition of a CH_2Cl_2 solution of **12**[21] (691 mg, 1.59 mmol) to the mixture at room temperature, the mixture was stirred for 0.5 h. The reaction mixture was washed with 1 M HCl, dried with Na_2SO_4, evaporated, and chromatographed (hexane/AcOEt 2:1) to yield **13** (902 mg, 94%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 1.25 (3H, t, J = 7.1 Hz), 1.28 (3H, t, J = 7.1 Hz), 1.34 (3H, d, J = 7.0 Hz), 1.39 (9H, s), 2.18–2.31 (1H, m), 2.32–2.54 (2H, m), 3.08 (1H, dd, J = 2.9 Hz, 7.9 Hz), 3.86–4.04 (1H, m), 4.06–4.16 (5H, m), 4.42 (1H, d, J = 11.6 Hz), 4.65 (1H, d, J = 11.6 Hz), 4.76–4.96 (1H, m), 7.06–7.24 (1H, m), 7.12 (1H, dd, J = 2.0 Hz, 8.1 Hz), 7.39 (1H, d, J = 2.0 Hz), 7.40 (1H, d, J = 8.1 Hz); MS (ion spray) (Negative) m/z 630 ($M-1)^-$; $[\alpha]_D^{24}$ −33.6 (c 0.42, $CHCl_3$).

5.1.5. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-((2*S*)-2-Aminopropionylamino)-3-(3,4-dichlorobenzyloxy)-6-fluoro-bicyclo[3. 1.0]hexane-2,6-dicarboxylic acid (7aa). A mixture of **13** (459 mg, 0.751 mmol), 2.5 M aqueous LiOH (6.0 mL, 15 mmol), and H_2O (6 mL) was stirred for 2 days at room temperature. The mixture was extracted three times with AcOEt, and the AcOEt layer was dried with Na_2SO_4 and concentrated under reduced pressure to obtain a colorless oil (470 mg). The oil was used for the next reaction without further purification, despite containing an unidentified impurity. A mixture of the oil (375 mg), $LiOH \cdot H_2O$ (135 mg, 3.21 mmol), and H_2O (7.5 mL) was stirred for 8 days at room temperature. The mixture was washed 10 times with AcOEt, acidified (pH 2) with 1 M HCl, and extracted with AcOEt. The AcOEt layer was washed with brine, dried with Na_2SO_4, and concentrated. Four normal solution of HCl/AcOEt (4.6 mL) was added to the residue, and the mixture was stirred for 15 h at room temperature. The obtained precipitate was filtrated, washed with AcOEt (5 mL), and dried under reduced pressure to yield **7aa** (138 mg, 61%) as a white powder. Mp > 260 (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 1.49 (3H, d, J = 6.8 Hz), 2.07–2.18 (1H, m), 2.21–2.38 (1H, m), 2.44–2.56 (1H, m), 2.75–2.83 (1H, m), 4.02–4.22 (2H, m), 4.50 (1H, d, J = 11.3 Hz), 4.71 (1H, d, J = 11.3 Hz), 7.29 (1H, d, J = 7.8 Hz), 7.50–7.58 (2H, m); MS (ion spray) (Negative) m/z 447 ($M-1)^-$; $[\alpha]_D^{26}$ −56.7 (c = 0.22, MeOH); Anal. Calcd for $C_{18}H_{19}Cl_2FNO_6 \cdot HCl$: C, 44.51; H, 4.15; N, 5.77. Found: C, 44.45; H, 4.12; N, 5.76.

5.1.6. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Azido-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-ethyl ester (14). One molar LiOH solution (2.23 mL) was added to a mixture of **11** (932 mg, 2.02 mmol), THF (16 mL), and H_2O (8 mL) with ice-cooling and stirred for 30 min. The mixture was acidified (pH 1) with 1 M HCl and extracted with AcOEt. The organic layer was washed with saturated brine, dried with Na_2SO_4, and evaporated to yield **14** (901 mg) as a brown colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 1.31 (3H, t, J = 7.0 Hz), 2.31–2.56 (4H, m), 3.90–4.05 (2H, m), 4.18–4.49 (3H, m), 4.65 (1H, d, J = 12.3 Hz), 7.10 (1H, dd, J = 8.1, 2.0 Hz), 7.35–7.44 (2H, m).

5.1.7. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Azido-3-(3,4-dichlorobenzyloxy)-6-((1*S*)-1-ethoxycarbonyl-3-methylbutylcarbamoyl)-6-fluorobicyclo[3.1.0]hexane-2-carboxylic acid ethyl ester (15). $HOBT \cdot H_2O$ (378 mg, 2.47 mmol) and $EDC \cdot HCl$ (455 mg, 2.37 mmol) were added to a DMF (8.5 mL) solution of **14** (854 mg, 1.98 mmol), L-leucine ethyl ester (464 mg, 2.37 mmol), and *N*-methylmorphrin (594 mL, 2.37 mmol) with ice-cooling. The mixture was stirred for 12 h at room temperature and diluted with AcOEt. The mixture was washed with 1 M HCl and saturated brine, dried with Na_2SO_4, evaporated, and chromatographed (hexane/AcOEt 8:1) to yield **15** (998 mg, 91%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 0.96 (6H, d, J = 5.5 Hz), 1.29 (3H, t, J = 7.0 Hz), 1.30 (3H, t, J = 7.0 Hz), 1.52–1.80 (3H, m), 2.26–2.57 (4H, m), 3.86–4.02 (1H, m), 4.22 (2H, q, J = 7.0 Hz), 4.10–4.38 (2H, m), 4.42 (1H, d, J = 12.2 Hz), 4.50–4.66 (1H, m), 4.65 (1H, d, J = 12.2 Hz), 6.79 (1H, d, J = 8.1 Hz), 7.11 (1H, dd, J = 8.1, 2.0 Hz), 7.38 (1H, d, J = 2.0 Hz), 7.40 (1H, d, J = 8.1 Hz); MS (ion spray) (Negative) m/z 571 ($M-1)^-$; $[\alpha]_D^{28}$ −20.0 (c 0.39, $CHCl_3$).

5.1.8. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-((1*S*)-1-ethoxycarbonyl-3-methylbutylcarbamoyl)-6-fluorobicyclo[3.1.0]hexane-2-carboxylic Acid Ethyl Ester (16). A mixture of **15** (996 mg, 1.74 mmol), 1 M Me_3P/THF (1.91 mL, 1.91 mmol), THF (25 mL), and H_2O (2.5 mL) was stirred for 5 h at room temperature. Et_2O and saturated $NaHCO_3$ were added to the mixture and stirred for 1 h at room temperature. The ethereal layer was separated and washed with saturated brine, dried with Na_2SO_4, and evaporated in vacuo. Silica gel and $CHCl_3$ were added to the obtained residue. The $CHCl_3$ was evaporated under reduced pressure, allowed to stand for 2 days, and chromatographed (hexane/AcOEt 3:2–1:1) to yield **16** (784 mg, 82%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ 0.96 (6H, d, J = 5.9 Hz), 1.29 (6H, t, J = 7.1 Hz), 1.52–1.77 (3H, m), 1.85 (2 H, s), 2.10–2.28 (2H, m), 2.36–2.48 (2H, m),

3.69–3.87 (1H, m), 4.21 (2H, q, J = 7.1 Hz), 4.15–4.36 (2H, m), 4.45 (1H, d, J = 12.1 Hz), 4.64 (1H, d, J = 12.1 Hz), 4.55–4.69 (1H, m), 6.77 (1H, dd, J = 8.0, 3.4 Hz), 7.10 (1H, dd, J = 8.4, 1.8 Hz), 7.38 (1H, d, J = 1.8 Hz), 7.39 (1H, d, J = 8.4 Hz); MS (ion spray) (Negative) m/z 545 $(M-1)^-$; $[\alpha]_D^{22}$ +2.4 (c 0.65, $CHCl_3$).

5.1.9. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-6-((1*S*)-1-carboxy-3-methylbutylcarbamoyl)-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2-carboxylic acid (7ab). A mixture of **16** (400 mg, 0.731 mmol), $LiOH{\cdot}H_2O$ (76.7 mg, 1.83 mmol), THF (8.4 mL), and H_2O (4.2 mL) was stirred for 24 h at room temperature. The mixture was diluted with H_2O, acidified (pH 1) with 1 M HCl, and chromatographed on AG50W-X8 cation-exchange resin (Bio-Rad) (H_2O–50% aqueous THF–10% aqueous pyridine) to yield **7ab** (250 mg, 59%) as a white powder. Mp > 190 °C. ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 0.89–0.94 (6H, m), 1.62–1.71 (3H, m), 2.37–2.39 (2H, m), 2.44–2.49 (1H, m), 2.55 (1H, dd, J = 14.0, 7.9 Hz), 4.10–4.14 (1H, m), 4.29–4.32 (1H, m), 4.54 (1H, d, J = 11.6 Hz), 4.61 (1H, d, J = 11.6 Hz), 5.30 (1H, d, J = 8.4 Hz), 7.55 (1H, d, J = 8.4 Hz), 7.56 (1H, s); MS (ion spray) (Negative) m/z 489 $(M-1)^-$; $[\alpha]_D^{25}$ −7.6 (c 0.46, MeOH); Anal. Calcd for $C_{21}H_{25}Cl_2FNO_6{\cdot}1.2H_2O$:C, 49.17; H, 5.38; N, 5.46. Found: C, 49.34; H, 5.66; N, 5.52.

5.1.10. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-ethyl ester (7ac). A mixture of **17** (150 mg, 0.354 mmol), $LiOH{\cdot}H_2O$ (17.8 mg, 0.425 mmol), THF (3.5 mL), and (H_2O (1.7 mL) was stirred for 2 h with ice-cooling. The mixture was acidified with 1 M HCl (0.45 mL), diluted with H_2O, and chromatographed on AG50W-X8 cation-exchange resin (Bio-Rad) (H_2O–50% aqueous THF–10% aqueous pyridine) to yield **7ac**[21] (107 mg, 75%) as a white powder. Mp > 223 °C; ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 1.31 (3H, t, J = 7.3 Hz), 2.26 (1H, dd, J = 7.9, 2.4 Hz), 2.30–2.33 (1H, m), 2.44–2.49 (1H, m), 2.55 (1H, dd, J = 13.4, 7.3 Hz), 4.08–4.12 (1H, m), 4.29–4.42 (2H, m), 4.51 (1H, d, J = 12.8 Hz), 4.54 (1H, d, J = 12.8 Hz), 7.22 (1H, d, J = 8.5 Hz), 7.49 (1H, d, J = 8.5 Hz), 7.50 (1H, s); MS (ion spray) (Negative) m/z 404 $(M-1)^-$; $[\alpha]_D^{28}$ −24.3 (c 0.46, MeOH); Anal. Calcd for $C_{21}H_{25}Cl_2FNO_6{\cdot}0.4H_2O$: C, 49.82; H, 4.53; N, 3.42. Found: C, 49.74; H, 4.65; N, 3.34.

5.1.11. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid 6-benzyl ester (7au). A mixture of **5** (400 mg, 1.06 mmol), $SOCl_2$ (0.31 mL, 4.26 mmol), and benzyl alcohol (6 mL) was stirred for 2.5 h at 50 °C. After cooling, benzyl alcohol was evaporated under reduced pressure. The residue was chromatographed on reverse-phase silica gel (Wakogel®50C18, H_2O–30% MeCN) to yield **7au** (240 mg, 48%). Mp > 193 °C (decomp.); ^{1}H NMR (200 MHz, CD_3OD, TMS) δ 2.30–2.61 (4H, m), 3.95–4.05 (1H, m), 4.46 (1H, d, J = 11.8 Hz), 4.58 (1H, d, J = 11.8 Hz), 5.23 (2H, s), 7.28 (1H, dd, J = 8.2, 1.9 Hz), 7.33–7.41 (5H, m), 7.45 (1H, d, J = 8.2 Hz), 7.53 (1H, d, J = 1.9 Hz); MS (ion spray) (Negative) m/z 466 $(M-1)^-$; $[\alpha]_D^{24}$ +5.3 (c 0.34, MeOH); Anal. Calcd for $C_{22}H_{20}Cl_2FNO_5$: C, 52.35; H, 4.19; N, 2.77. Found: C, 52.10; H, 4.40; N, 2.75.

5.1.12. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid 6-methyl ester (7ad). A mixture of **5** (114 mg, 0.301 mmol), $SOCl_2$ (88 μL, 1.21 mmol), and MeOH (1.1 mL) was stirred for 1 h at 50 °C. After cooling, MeOH was evaporated under reduced pressure. Diisopropyl ether (IPE) was added to the residue, and the obtained precipitate was filtered to yield **7ad** (102 mg, 79%) as a white powder. Mp > 190 °C (decomp.); ^{1}H NMR (200 MHz, CD_3OD, TMS) δ 2.37–2.63 (4H, m), 3.81 (3H, s), 4.03–4.18 (1H, m), 4.55 (2H, s), 7.26 (1H, dd, J = 8.3, 1.8 Hz), 7.48 (1H d, J = 8.3 Hz), 7.53 (1H, d, J = 1.8 Hz); MS (ion spray) (Negative) m/z 390 $(M-1)^-$; $[\alpha]_D^{30}$ minus13.2 (c = 0.64, MeOH); Anal. Calcd for $C_{16}H_{16}Cl_2FNO_5{\cdot}HCl{\cdot}1.5H_2O$: C, 42.17; H, 4.42; N, 3.07. Found: C, 42.42; H, 4.46; N, 3.01.

5.1.13. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid ethyl ester (7ae). Mp > 158 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 1.29 (3H, t, J = 6.9 Hz), 2.34–2.50 (2H, m), 2.52–2.68 (2H, m), 4.05–4.18 (1H, m), 4.20–4.34 (1H, m), 4.48–4.63 (1H, m), 7.15–7.20 (1H, m), 7.47 (1H, d, J = 7.3 Hz), 7.52 (1H, s); MS (ion spray) (Negative) m/z 404 $(M-1)^-$; $[\alpha]_D^{29}$ −8.1 (c = 0.24, MeOH); Anal. Calcd for $C_{17}H_{18}Cl_2FNO_5{\cdot}HCl{\cdot}0.5H_2O$: C, 45.20; H, 4.46; N, 3.10. Found: C; 45.34, H, 4.51; N, 2.99.

5.1.14. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid ethyl ester propyl ester (7af). Mp > 149 °C (decomp.); ^{1}H NMR (200 MHz, CD_3OD, TMS) δ 0.92–1.00 (3H, m), 1.69 (2H, sextet, J = 6.8 Hz), 2.37–2.65 (4H, m), 4.02–4.12 (1H, m), 4.17 (2H, t, J = 6.6 Hz), 4.51 (1H, d, J = 11.9 Hz,), 4.59 (1H, d, J = 11.9 Hz), 7.27 (2H, dd, J = 8.4, 1.8 Hz), 7.48 (1H, d, J = 8.4 Hz), 7.54 (1H, d, J = 1.8 Hz); MS (ion spray) (Negative) m/z 418 $(M-1)^-$; $[\alpha]_D^{28}$ +7.8 (c 0.35, MeOH) as the salt free form; Anal. Calcd for $C_{18}H_{20}Cl_2FNO_5$: C, 51.44; H, 4.80; N, 3.33. Found: C, 51.40; H, 4.82; N, 3.30.

5.1.15. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid iso-propyl ester (7ag). Mp > 161 °C (decomp.); ^{1}H NMR (500 MHz, CD_3OD, TMS) δ 1.28 (6H, d, J = 5.8 Hz), 2.38–2.44 (2H, m), 2.54–2.62 (2H, m), 4.07–4.12 (1H, m), 4.53 (1H, d, J = 11.6 Hz), 4.57 (1H, d, J = 11.6 Hz), 5.07–5.12 (1H, m), 7.26 (1H, dd, J = 1.8, 8.6 Hz), 7.48 (1H, d, J = 8.6 Hz), 7.52 (1H, d, J = 1.8 Hz); MS (ion spray) (Negative) m/z 418 $(M-1)^-$; $[\alpha]_D^{28}$ +10.4 (c 0.25, MeOH) as the salt free form; Anal. Calcd for $C_{18}H_{20}Cl_2FNO_5{\cdot}HCl$: C, 47.34; H, 4.63; N, 3.07. Found: C, 47.41; H, 4.90; N, 3.11.

5.1.16. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid butyl ester (7ah). Mp > 146 °C (decomp.); ^{1}H NMR (500 MHz, CD_3OD, TMS) δ 0.95 (3H, t,

J = 7.9 Hz), 1.40 (2H, sextet, J = 7.9 Hz), 1.65 (2H, quintet, J = 7.9 Hz), 2.38–2.44 (2H, m), 2.53–2.62 (2H, m), 4.06–4.10 (1H, m), 4.22 (2H, t, J = 6.7 Hz), 4.52 (1H, d, J = 11.6 Hz), 4.58 (1H, d, J = 11.6 Hz), 7.26 (1H, dd, J = 1.8, 7.9 Hz), 7.47 (1H, d, J = 7.9 Hz) 7.53 (1H, d, J = 1.8 Hz); MS (ion spray) (Negative) m/z 432 $(M-1)^-$; $[\alpha]_D^{28}$ +10.4 (c 0.25, MeOH) as the salt free form; Anal. Calcd for $C_{19}H_{22}Cl_2FNO_5 \cdot 0.5HCl$: C, 51.48; H, 5.23; N, 3.16. Found: C, 51.27; H, 5.20; N, 3.06.

5.1.17. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid 3-methylbutyl ester (7ai). Mp > 158 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 0.95 (6H, d, J = 6.7 Hz), 1.90–2.03 (1H, m), 2.35–2.66 (4H, m), 4.00 (2H, d, J = 6.5 Hz), 4.03–4.20 (1H, m), 4.52 (1H, d, J = 10.7 Hz), 4.58 (1H, d, J = 10.7 Hz), 7.26 (1H, dd, J = 8.2, 1.9 Hz), 7.47 (1H, d, J = 8.2 Hz), 7.53 (1H, d, J = 1.9 Hz); MS (ion spray) (Negative) m/z 432 $(M-1)^-$; $[\alpha]_D^{30}$ −7.9 (c 0.67, MeOH); Anal. Calcd for $C_{22}H_{26}Cl_2FNO_5 \cdot HCl$: C, 51.73; H, 5.33; N, 2.74. Found: C, 52.13; H, 5.61; N, 2.70.

5.1.18. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid pentyl ester (7aj). Mp > 148 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 0.93 (3H, t, J = 7.3 Hz), 1.34–1.37 (2H, m), 1.65–1.71 (2H, m), 2.39–2.45 (2H, m), 2.55–2.60 (2H, m), 4.08–4.12 (1H, m), 4.03 (2H, t, J = 6.7 Hz), 4.53 (1H, d, J = 12.2 Hz), 4.58 (1H, d, J = 12.2 Hz), 7.26 (1H, dd, J = 8.6, 1.8 Hz), 7.48 (1H, d, J = 8.6 Hz), 7.53 (1H, d, J = 1.8 Hz); MS (ion spray) (Negative) m/z 446 $(M-1)^-$; $[\alpha]_D^{30}$ −8.6 (c 0.59, MeOH); Anal. Calcd for $C_{20}H_{24}Cl_2FNO_5$: C, 53.58; H, 5.40; N, 3.12. Found: C, 53.27; H, 5.42; N, 3.08.

5.1.19. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid cyclohexyl ester (7an). Mp > 167 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 1.27–1.89 (10H, m), 2.32–2.64 (4H, m), 3.95–4.08 (1H, m) 4.47 (1H, d, J = 12.0 Hz), 4.59 (1H, d, J = 12.0 Hz), 7.28 (1H, dd, J = 8.2, 1.9 Hz), 7.45 (1H, d, J = 8.24 Hz), 7.54 (1H, d, J = 1.86 Hz); MS (ion spray) (Negative) m/z 458 $(M-1)^-$; $[\alpha]_D^{30}$ −5.3 (c 0.11, MeOH); Anal. Calcd for $C_{22}H_{26}Cl_2FNO_5 \cdot HCl$: C, 54.79; H, 5.26; N, 3.04. Found: C, 54.69; H, 5.30; N, 3.05.

5.1.20. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid cyclohexylmethyl ester (7aq). Mp > 153 °C (decomp.); ^{1}H NMR (500 MHz, CD_3OD, TMS) δ 0.97–1.05 (2H, m), 1.16–1.33 (3H, m), 1.63–1.77 (6H, m), 2.40–2.46 (2H, m), 2.58–2.60 (2H, m), 4.03 (2H, d, J = 6.7 Hz), 4.09–4.13 (1H, m), 4.54 (1H, d, J = 11.6 Hz), 4.58 (1H, d, J = 11.6 Hz), 7.26 (1H, dd, J = 1.8, 8.6 Hz), 7.48 (1H, d, J = 8.6 Hz), 7.52 (1H, d, J = 1.8 Hz); MS (ion spray) (Negative) m/z 472 $(M-1)^-$; $[\alpha]_D^{30}$ −6.9 (c 0.47, MeOH); Anal. Calcd for $C_{17}H_{18}Cl_2FNO_5 \cdot HCl$: C, 46.12; H, 4.33; N, 3.16. Found: C, 46.07; H, 4.61; N, 3.22.

5.1.21. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-Dichlorobenzyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid ethyl ester (7az). Mp > 188 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 1.24 (3H, t, J = 7.1 Hz), 1.97–2.01 (1H, m), 2.15–2.51 (4H, m), 3.76–3.80 (1H, m), 4.13 (2H, q, J = 7.2 Hz), 4.50 (2H, s), 7.24 (1H, dd, J = 8.2, 1.9 Hz), 7.48 (1H, d, J = 8.2 Hz), 7.51 (1H, d, J = 1.9 Hz); MS (ion spray) (Negative) m/z 386 $(M-1)^-$; $[\alpha]_D^{30}$ −1.4 (c 0.13, MeOH); Anal. Calcd for $C_{17}H_{19}Cl_2NO_5 \cdot 0.9HCl$: C, 48.49; H, 4.76; N, 3.33. Found: C, 48.77; H, 4.91; N, 3.31.

5.1.22. Typical procedure 2

5.1.22.1. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]- hexane-2,6-dicarboxylic acid 6-(3-methyl)butyl ester (7ak). A mixture of **5** (50 mg, 0.132 mmol), $SOCl_2$ (35 mL, 0.481 mmol), and 3-methylbutan-1-ol (1 mL) was stirred for 2.5 h at 60 °C. After cooling, the mixture was concentrated under reduced pressure. Propylene oxide (0.5 mL) and EtOH (0.5 mL) were added to the residue, and the mixture was refluxed for 45 min. The obtained precipitate was filtered and washed with Et_2O, IPE, and hexane to yield **7ak** (43 mg, 73%) as a white powder. Mp > 235 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 0.93 (6H, d, J = 6.5 Hz), 1.52–1.59 (2H, m), 1.65–1.74 (1H, m), 2.31–2.64 (4H, m), 3.97–4.05 (1H, m), 4.24 (2H, t, J = 6.7 Hz), 4.47 (1H, d, J = 12.1 Hz), 4.59 (1H, d, J = 12.1 Hz), 7.28 (1H, dd, J = 8.2, 2.0 Hz), 7.45 (1H, d, J = 8.2 Hz), 7.54 (1H, d, J = 2.0 Hz); MS (ion spray) (Negative) m/z 446 $(M-1)^-$; $[\alpha]_D^{30}$ +9.2 (c 0.47, MeOH); Anal. Calcd for $C_{20}H_{24}Cl_2FNO_5$: C, 53.58; H, 5.40; N, 3.12. Found: C, 53.55; H, 5.36; N, 3.14.

5.1.22.2. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-Dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid hexyl ester (7al). Mp > 200 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 0.91 (3H, t, J = 6.7 Hz), 1.26–1.43 (6H, m), 1.60–1.72 (2 H, m), 2.31–2.67 (4H, m), 3.97–4.06 (1H, m), 4.19 (2H, t, J = 6.6 Hz), 4.47 (1H, d, J = 12.0 Hz), 4.59 (1H, d, J = 12.0 Hz), 7.28 (1H, dd, J = 8.2, 1.7 Hz), 7.45 (1H, d, J = 8.2 Hz), 7.54 (1H, d, J = 1.7 Hz); MS (ion spray) (Negative) m/z 460 $(M-1)^-$; $[\alpha]_D^{27}$ +5.0 (c = 0.57, MeOH); Anal. Calcd for $C_{21}H_{26}Cl_2FNO_5$: C, 54.55; H, 5.67; N, 3.03. Found: C, 54.59; H, 5.68; N, 3.06.

5.1.22.3. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid 4-methylpentyl ester (7am). Mp > 198 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 0.91 (6H, d, J = 6.7 Hz), 1.19–1.31 (2H, m), 1.49–1.72 (3H, m), 2.32–2.66 (4H, m), 3.98–4.70 (1H, m), 4.18 (1H, t, J = 6.6 Hz), 4.48 (1H, d, J = 11.8 Hz), 4.60 (1H, d, J = 11.8 Hz), 7.29 (1H, dd, J = 8.2, 2.1 Hz), 7.46 (1H, d, J = 8.2 Hz), 7.55 (1H, d, J = 2.1 Hz); MS (ion spray) (Positive) m/z 462 $(M+1)^+$; $[\alpha]_D^{28}$ +7.0 (c 0.41, MeOH); Anal. Calcd for $C_{21}H_{26}Cl_2FNO_5$: C, 54.55; H, 5.67; N, 3.03. Found: C, 54.52; H, 5.68; N, 3.00.

5.1.22.4. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid heptyl ester (7ao). Mp > 214 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 0.90 (3H, t, J = 6.5 Hz), 1.26–1.42 (8H, m), 1.59–1.72 (2H, m), 2.31–2.40 (2H, m), 2.48 (1H, dd, J = 13.3, 7.4 Hz), 2.50–2.66 (1H, m), 3.98–4.03 (1H, m), 4.19 (2H, t, J = 6.5 Hz), 4.47 (1H, d, J = 12.0 Hz), 4.59 (1H, d, J = 12.0 Hz), 7.28 (1H, dd, J = 8.2 Hz), 7.45 (1H, d, J = 8.2 Hz), 7.54 (1H, d, J = 2.0 Hz); MS (ion spray) (Positive) m/z 476 $(M+1)^+$; $[\alpha]_D^{27}$ +8.2 (c 0.49, MeOH); Anal. Calcd for $C_{22}H_{28}Cl_2FNO_5$: C, 55.47; H, 5.92; N, 2.94. Found: C, 55.37; H, 5.90; N, 2.76.

5.1.22.5. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid 4-methylpentyl ester (7ap). Mp > 209 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 0.90 (6H, d, J = 6.5 Hz), 1.17–1.71 (7H, m), 2.32–2.40 (2H, m), 2.49 (1H, dd, J = 13.2, 7.3 Hz), 2.55–2.66 (1H, m), 3.98–4.07 (1H, m), 4.20 (2H, t, J = 6.5 Hz), 4.48 (1H, d, J = 12.0 Hz), 4.60 (1H, d, J = 12.0 Hz), 7.29 (1H, dd, J = 8.2, 1.9 Hz), 7.46 (1H, d, J = 8.2 Hz), 7.55 (1H, d, J = 1.9 Hz); MS (ion spray) (Positive) m/z 476 $(M+1)^+$; $[\alpha]_D^{30}$ +4.9 (c 0.48, MeOH); Anal. Calcd for $C_{22}H_{28}Cl_2FNO_5$: C; 55.47; H, 5.92; N, 2.94. Found: C, 55.30; H, 5.99; N, 2.92.

5.1.22.6. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid octyl ester (7ar). Mp > 203 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 0.90 (3H, t, J = 6.6 Hz), 1.25–1.42 (10H, m), 1.60–1.71 (2H, m), 2.31–2.40 (2H, m), 2.48 (1H, dd, J = 13.4, 7.6 Hz), 2.5–2.7 (1H, m), 4.05–4.06 (1H, m), 4.19 (2H, t, J = 6.6 Hz), 4.47 (1H, d, J = 12.0 Hz), 4.59 (1H, d, J = 12.0 Hz), 7.28 (1H, dd, J = 8.2, 1.9 Hz), 7.45 (1H, d, J = 8.2 Hz), 1.9 (1H, d, J = 1.9 Hz); MS (ion spray) (Positive) m/z 490 $(M+1)^+$; $[\alpha]_D^{30}$ +6.3 (c 0.52, MeOH); Anal. Calcd for $C_{23}H_{30}Cl_2FNO_5$: C, 56.33; H, 6.17; N, 2.86. Found: C, 56.11; H, 6.18; N, 2.86.

5.1.22.7. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid 6-methylheptyl ester (7as). Mp > 193 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 0.89 (6H, d, J = 6.7 Hz), 1.15–1.39 (6H, m), 1.47–1.71 (3H, m), 2.33–2.65 (4H, m), 4.02 (1H, m), 4.19 (2H, t, J = 6.5 Hz), 4.48 (1H, d, J = 12.1 Hz), 4.60 (1H, d, J = 12.1 Hz), 7.29 (1H, dd, J = 8.2, 2.0 Hz), 7.46 (1H, d, J = 8.2 Hz), 7.54 (1H, d, J = 2.0 Hz); MS (ion spray) (Negative) m/z 488 $(M-1)^-$; $[\alpha]_D^{30}$ +4.7 (c 0.53, MeOH); Anal. Calcd for $C_{23}H_{30}Cl_2FNO_5$: C, 56.33; H, 6.17; N, 2.86. Found: C, 56.09; H, 6.04; N, 2.87.

5.1.22.8. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid decyl ester (7at). Mp > 196 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 2.30–2.61 (4H, m), 3.95–4.05 (1H, m), 4.46 (1H, d, J = 11.8 Hz), 4.58 (1H, d, J = 11.8 Hz), 5.23 (2H, s), 7.28 (1H, dd, J = 8.2, 1.9 Hz), 7.33–7.41 (5H, m), 7.45 (1H, d, J = 8.2 Hz), 7.53 (1H, d, J = 1.9 Hz); MS (ion spray) (Negative) m/z 466 $(M-1)^-$; $[\alpha]_D^{27}$ +10.5 (c 0.52, MeOH); Anal. Calcd for $C_{25}H_{34}Cl_2FNO_5$: C, 57.92; H, 6.61, N, 2.70. Found: C, 57.85; H, 6.60; N, 2.72.

5.1.22.9. (1′*R*,2′*R*,3′*R*,5′*R*,6′*R*)-3′-(3,4-Dichlorobenzyloxy)-6′-fluoro-3-allyloxycarbonyl-5-oxo-oxazinone-4-spiro-2′-bicyclo[3.1.0]hexane-6′-carboxylic acid (17). A mixture of **5** (1.55g, 3.97 mmol), allyl chloroformate (957 mg, 7.94 mmol), saturated $NaHCO_3$ (13 mL), and dioxane (5.3 mL) was stirred for 18 h at room temperature. The mixture was diluted with H_2O (5 mL) and washed with AcOEt. The aqueous layer was acidified (pH 1) with 1 N HCl and extracted with AcOEt. The AcOEt layer was washed with H_2O, dried with Na_2SO_4, and evaporated under reduced pressure to obtain a white foam. A mixture of the white foam, paraformaldehyde (503 mg), $TsOH{\cdot}H_2O$ (36 mg, 0.189 mmol), and benzene was refluxed for 6 h using Dean–Stark apparatus. After cooling, the mixture was diluted with AcOEt, washed with H_2O, dried with Na_2SO_4, and evaporated under reduced pressure to yield **17** (1.67 g, 89%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 2.07–2.54 (m, 4H), 4.17–4.24 (1H, m), 4.39 (1H, d, J = 12.3 Hz), 4.52 (1H, d, J = 12.3 Hz), 4.63 (2H, d, J = 6.2 Hz), 5.23 (1H, d, J = 4.4 Hz), 5.28–5.54 (2H, m), 5.53 (1H, d, J = 4.5 Hz), 5.85–5.98 (1H, m), 7.07 (1H, dd, J = 8.2, 1.9 Hz), 7.32 (1H, d, J = 1.9 Hz), 7.41 (1H, d, J = 8.2 Hz); MS (ion spray) (Negative) m/z 472 $(M-1)$.

5.1.22.10. (1′*R*,2′*R*,3′*R*,5′*R*,6′*R*)-3′-(3,4-Dichlorobenzyloxy)-6′-fluoro-3-allyloxycarbonyl-5-oxo-oxazinone-4-spiro-2′-bicyclo[3.1.0]hexane-6′-carboxylic acid 1-ethoxycarbonyloxyethyl ester (18av). A mixture of **17** (250 mg, 0.527 mmol), 1-chloroethyl ethyl carbonate (193 mg, 1.27 mmol), NaI (190 mg, 1.27 mmol), K_2CO_3 (87 mg, 0.633 mmol), and DMF was stirred for 3 h at 75 °C. After the mixture was stirred for 12 h at room temperature, AcOEt was added to the mixture. The AcOEt layer was washed with H_2O and saturated brine, dried with Na_2SO_4, evaporated, and chromatographed (hexane/AcOEt 5:1) to yield **18av** as a pale yellow oil.

5.1.22.11. (1*R*,2*R*,3*R*,5*R*,6*R*)2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-carboxylic acid 1-ethoxycarbonyloxyethyl ester (7av). A mixture of **18av** (168 mg, 0.285 mmol), $Pd(PPh_3)_4$ (10 mg, 8.5 mmol), 1,3-dimethylbarbituric acid (133 mg, 0.854 mmol), and $CHCl_3$ (2.9 mL) was stirred for 1.5 h at 40 °C under nitrogen atmosphere. The mixture was concentrated under reduced pressure. AcOEt was added to the residue and stirred for 1 h at room temperature. After the mixture was stirred for 1 h with ice cooling, the obtained precipitate was filtrated off, after which the filtrate was evaporated under reduced pressure. The obtained residue was purified using reverse-phase silica gel (Wakogel®50C18, H_2O–40% MeCN) to yield **7av** (118 mg, 84%). A white powder. Mp ≥126 °C (decomp.); ^{1}H NMR (200 MHz, CD_3OD, TMS) δ 2.37–2.63 (4H, m), 3.81 (3H, s), 4.03–4.18 (1H, m), 4.55 (2H, s), 7.26 (1H, dd, J = 8.3, 1.8 Hz), 7.48 (1H d, J = 8.3 Hz), 7.53 (1H, d, J = 1.8 Hz); MS (ion spray) (Negative) m/z 390 $(M-1)$; Anal. Calcd for

$C_{16}H_{16}Cl_2F_2NO_5 \cdot HCl \cdot 0.5H_2O$: C, 47.73; H, 4.61; N, 2.78. Found: C, 47.73; H, 4.47; N, 2.81.

5.1.22.12. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid 1-cyclohexyloxycarbonyloxyethyl ester (7aw). Mp > 133 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 1.21–1.61 (8H, m), 1.68–1.96 (4H, m), 2.30–2.66 (4H, m), 3.98–4.07 (1H, m), 4.47 (1H, d, J = 12.0 Hz), 4.57–4.67 (2H, m), 6.76 (1H, q, J = 5.3 Hz), 7.28 (1H, dd, J = 8.3, 2.0 Hz), 7.45 (1H, d, J = 8.3 Hz), 7.54 (1H, d, J = 2.0 Hz); MS (ion spray) (Negative) m/z 458 $(M-1)^-$; Anal. Calcd for $C_{24}H_{28}Cl_2FNO_8 \cdot 0.2H_2O$: C, 52.22; H, 5.19; N, 2.54. Found: C, 52.03; H, 5.17; N, 2.46.

5.1.22.13. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid butanoyloxymethyl ester (7ax). Mp > 152 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 0.95 (3H, t, J = 7.4 Hz), 1.64 (2H, sextet, J = 7.4 Hz), 2.31–2.97 (6H, m), 3.97–4.06 (1H, m), 4.47 (1H, d, J = 12.1 Hz), 4.59 (2H, d, J = 12.1 Hz), 5.83 (2H, s), 7.28 (1H, dd, J = 8.2, 2.0 Hz), 7.45 (1H, d, J = 8.2 Hz), 7.54 (1H, d, J = 2.0 Hz); MS (ion spray) (Negative) m/z 476 $(M-1)^-$; $[\alpha]_D^{30}$ +10.5 (c = 0.53, MeOH); Anal. Calcd for $C_{20}H_{22}Cl_2FNO_7 \cdot 0.7H_2O$: C, 48.93; H, 4.80; N, 2.85. Found: C, 48.97; H, 4.77; N, 2.85.

5.1.22.14. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-(morphrin-4-yl)ethyl ester (7ay). Mp > 190 °C (decomp.); ^{1}H NMR (300 MHz, D_2O, TMSP) δ 2.47–1.58 (4H, m), 3.33–3.41 (4H, m), 3.52–3.58 (2H, m), 3.92–4.13 (5H, m), 4.48–4.64 (4H, m), 7.29 (1H, dd, J = 8.2, 1.9 Hz), 7.53–756 (2H, m); MS (ion spray) (Negative) m/z 489 $(M-1)^-$; $[\alpha]_D^{30}$ +3.5 (c 0.31, MeOH); Anal. Calcd for $C_{21}H_{25}Cl_2FN_2O_6 \cdot 2HCl \cdot H_2O$: C, 43.32; H, 5.02; N, 4.81. Found: C, 43.48; H, 4.87; N, 4.88.

5.1.22.15. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)bicyclo[3.1.0]hexane-2,6-carboxylic acid 1-ethoxycarbonyloxyethyl ester (7ba). Mp > 194 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 1.22–1.32 (3H, m), 1.42–1.50 (3H, m), 1.87–1.94 (1H, m), 2.11–2.27 (2H, m), 2.32–2.41 (2H, m), 3.61–3.70 (1H, m), 4.11–4.23 (2H, m), 4.44 (1H, d, J = 12.0 Hz), 4.51 (1H, d, J = 12.0 Hz), 6.63–6.72 (1H, m), 7.22–7.30 (1H, m), 7.45 (1H, d, J = 8.24 Hz), 7.53 (1H, s); MS (ion spray) (Negative) m/z 474 $(M-1)^-$; Anal. Calcd for $C_{20}H_{23}Cl_2NO_8 \cdot H_2O$: C, 49.50; H, 4.98; N, 2.89. Found: C, 49.46; H, 4.88; N, 2.85.

5.1.22.16. (1*R*,2*R*,3*R*,5*R*,6*R*)-2-Amino-3-(3,4-dichlorobenzyloxy)bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 1-cyclohexyloxycarbonyloxyethyl ester (7bb). Mp > 198 °C (decomp.); ^{1}H NMR (300 MHz, CD_3OD, TMS) δ 1.30–1.98 (14H, m), 2.11–2.27 (2H, m), 2.31–2.42 (2H, m), 3.59–3.71 (1H, m), 4.44 (1H, d, J = 12.1 Hz), 4.51 (1H, d, J = 12.1 Hz), 4.54–4.66 (1H, m), 6.61–6.72 (1H, m), 7.26 (1H, dd, J = 8.2, 1.7 Hz), 7.45 (1H, d, J = 8.2 Hz), 7.53 (1H, d, J = 1.7 Hz); MS (ion spray) (Negative) m/z 528 $(M-1)^-$; Anal. Calcd for $C_{24}H_{29}Cl_2NO_8 \cdot H_2O$: C, 53.63; H, 5.42; N, 2.7. Found: C, 53.44; H, 5.61; N, 2.60.

5.2. Metabolic stability study

Liver S9 fractions (1 mg protein/mL) from rat (Gentest) were incubated with 3μM prodrugs in the presence of an NADPH generating system (125 μg/mL $NADP^+$, 2.5 mM $MgCl_2$, and 1.92 mM glucose-6-phosphate) in 0.255 M phosphate buffer containing 0.575% (w/v) KCl (pH 7.4) for 60 min at 37 °C. All experiments were performed in triplicate. After incubation, a 2-fold volume of DMSO was added to incubation medium, and the tube was vortexed and centrifuged at 11,200g (4 °C) for 10 min. The resulting supernatant was analyzed with a LC–MS/MS system.

Rat plasma were spiked with prodrugs at concentrations of 1000 ng/mL (approximately 2.3 μM) and then incubated for 2 h at 37 °C. 200 μL of I.S. working solution (250 ng/mL) with methanol or acetonitrile was added to 50 μL aliquot of plasma sample, and the tube was vortexed and centrifuged at 11,200g (4 °C) for 10 min. The resulting supernatant was analyzed by a LC–MS/MS system.

5.2.1. Pharmacokinetics. *Animals*. The experiments used 7-week-old Male Wistar or Sprague–Dawley rats (Charles River, Japan). All animals in these experiments were used following acclimation for at least 4 days before entry into a study. The rats were given access to water and administered a standard laboratory diet (MF, Oriental Yeast Co, Japan) ad libitum during acclimation. The environmental parameters during breeding were maintained at relative humidity 50 ± 20% and temperature 23 ± 3 °C. The animals were fasted overnight (about 18 h) before and 4 h after dosing when studies were conducted in the fasting state. Drinking water was made freely available at all times.

5.2.2. Plasma concentrations. In the case of rats, 0.2–0.3 mL blood samples were taken from the tail vein using a Multivette containing EDTA, or from the jugular vein using a syringe, and then transferred to a Multivette at 0.5, 1, 2, 4 and 6 h post-dosing. For mice, after tail suspension test, animals were immediately euthanized under anesthesia by ether, and blood samples were taken via the femoral vein with tube (containing EDTA). Plasma was separated by centrifugation (11,200g, 4 °C, 2–3 min). In the case of prodrugs, plasma was immediately mixed with 5 N HCl (plasma/5 N HCl 50:1) to avoid enzymatic degradation in plasma. The plasma samples were immediately frozen with liquid N_2 and stored at −80 °C until bioanalysis.

To a 50 μL aliquot of a plasma sample, 200 μL of I.S. working solution (250 ng/mL) with methanol or acetonitrile was added, and the tube was vortexed and centrifuged at 11,200g (4 °C) for 10 min. The resulting supernatant was analyzed by a LC–MS/MS system.

5.2.3. Brain concentrations. After blood sampling at 6 h, animals were decapitated at the specified time. The brain (cerebrum) was immediately excised and weighed, and dura mater removed with soft paper. Each tissue was added 4-fold distilled water and homogenized.

To a 50 μL aliquot of a plasma or tissue homogenate sample, 200 μL of I.S. working solution (250 ng/mL) with methanol or acetonitrile was added, and the tube was vortexed and centrifuged at 11,200*g* (4 °C) for 10 min. The resulting supernatant was analyzed by s LC–MS/MS system.

5.3. Antidepressant-like effects

5.3.1. Animals. Male ICR mice (25–35 g, Charles River, Yokohama, Japan) were used for tail suspension test and locomotor activity. All of these animals were maintained under a 12 h light/dark cycle (light on 7:00 AM) in a temperature- and humidity-controlled holding room. Food and water were available ad libitum.

5.3.2. Ethics. All studies were reviewed by the Taisho Pharmaceutical Co., Ltd. Animal Care Committee and met the Japanese Experimental Animal Research Association standards, as defined in *The Guidelines for Animal Experiments* (1987).

5.3.3. Forced swimming test in rats. Forced swimming tests were performed by the method previously reported, and the effect of the compound was evaluated by measuring the period of immobility and by a time-sampling technique. A time-sampling technique was used to score several types of behavior (immobility, swimming, and climbing) as described by Derke et al.[42] Swimming sessions were conducted by placing rats in cylinders containing 25 °C water at a depth of 30 cm, sufficient to prevent rats from keeping their heads above water by standing on the bottom. Two swimming sessions were conducted; an initial 15 min pretest between 10:00 AM and 4:00 PM, followed 24 h later by a 5 min test. Compound **8a** was administered po during the period between these two sessions (24 and 2 h prior to the test). Following both swimming sessions, the rats were removed from the cylinders, placed in a heated cage for 15 min, and then returned to their home cages. Test sessions were videotaped from the front of the cylinders for later scoring. The water in the cylinders was changed after every trial. A time-sampling technique was used to score behavior during a single viewing: At the end of each 5-s period during the test session, the scorer, who remained unaware of the drug administered, classified the rat's behavior into one the following three categories: (1) immobility-floating in the water without struggling and making only movements necessary to keep its head above water; (2) swimming-making active swimming motions between quadrants of the cylinder, beyond those necessary merely to maintain head above water and moving around in the cylinder; and (3) climbing movements with forepaws in and out of the water, usually directed against the walls of the cylinder.

5.3.4. Tail suspension test in mice. Tail suspension tests were performed by the method described by Steru et al., with modification. Mice were suspended by the tail using adhesive tape from a metal rod fixed 45 cm above the surface of a table in a sound-isolated room. The mouse was positioned at least 15 cm away from the nearest object. Test sessions were videotaped for 10 min, and the immobility time was determined by an observer. Mice were considered immobile only when they hung passively and completely motionless. Compound **8ao** was administered po 2 h prior to the test.

5.3.5. Spontaneous locomotor activity. Spontaneous locomotor activity was determined as reported.[22] Animals were individually housed in transparent acrylic cages (for rats, $45 \times 28.5 \times 29.5$ cm; for mice, 30 cm diameter, 30 cm height), and spontaneous locomotor activity was recorded every 5 min for 60 min, using a SCANET apparatus (Neuroscience Inc., Japan) placed in a sound-proof box. Compound **8ao** was administered po 2 h before the start of measurement.

5.3.6. Statistical analysis. Data from in vivo experiments were analyzed by one-way ANOVA. Significant differences between groups were determined by Dunnett's test.

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